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Ultra-sensitive electrical immunoassay biosensors using nanotextured zinc oxide thin films on printed circuit board platforms

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ABSTRACT

This study demonstrates the development of nanotextured zinc oxide (ZnO) thin films sputter deposited on printed circuit boards (PCB) to enhance the capability in detecting low concentrations of the protein troponin-T. The presence of this particular biomarker in the bloodstream is a direct indicator of current and/or future risk of various forms of cardiovascular diseases. Electrical transduction through impedance spectroscopy was used to detect troponin-T functionalized immunoassays on nanotextured ZnO surfaces. Calibration of the immunoassay was performed by measuring the impedance changes resulting from the binding of increasing concentrations of troponin-T to the immobilized antibodies on the ZnO surface in (i) phosphate buffered saline (PBS) and (ii) human serum. The limit of detection achieved using this platform was 10 fg/mL and 100 fg/mL in PBS and human serum, respectively. Enhanced detection of troponin-T was found to correlate to the oxygen vacancies in the ZnO thin film. PCB was chosen as the substrate for ease of integration with microelectronic device manufacturing.

1. Introduction

Zinc oxide (ZnO) is used extensively for applications in gas sensing and removal, however lately it has gained considerable interest in the field of biosensing (Spencer, 2012). Current research in biomedical applications is focused mainly on the quantification of either glucose or cholesterol through detection of products resulting from enzymatic reactions. Various transduction mechanisms have been utilized to identify these biomolecules including electrical, optical, and piezoelectric-based methods (Fu et al., 2010; Singh et al., 2007; Wang et al., 2006). The wide range of sensing techniques that can be exploited using ZnO are due to its unique physical and chemical properties, including a wide band gap, large exciton binding energy, transparency, electron mobility, and biocompatibility (Jagadish and Pearton, 2011).

Electrical biosensing is shown to be more robust than other label-free transduction mechanisms due to its speed, sensitivity, ease-of-use, and low cost (Zhang et al., 2011). The electrical and material properties of ZnO make it ideal for this type of application. Native defects such as vacancies and interstitials can be modulated to alter the conductivity of ZnO (Janotti and Walle, 2009). Incorporation of dopants in ZnO can be used to alter the semiconducting nature of the material (Koßmann and Hattig, 2012). ZnO has a columnar wurtzite structure and growth along the *c*-axis results in either zinc or oxygen terminated surfaces, creating polar (0001) and (000) planes respectively. The (110) and (100) planes, on the other hand, are non-polar due to the equivalent amount of each atom (Wöll, 2007). This type of crystal structure allows for creation of columnar ZnO thin films with inherently nanotextured surfaces (van de Pol et al., 1991). Surfaces such as these have been found to be beneficial for biosensing because they can improve molecular binding as well as enhance the device's detection capabilities (Kunduru et al., 2010; Rath et al., 2012).

The biomolecule chosen for the experiments is a well-established cardiac biomarker, troponin-T, whose detection in the bloodstream signifies acute myocardial damage. Patients suffering from a myocardial infarction or other debilitating cardiovascular ailment will show concentrations of troponin-T in the picogram (pg) to nanogram per milliliter (ng/mL) range. Current technologies on the market claim to achieve detection limits as low as 10 pg/mL, however there is an increase in data variance at these lower limits (Hermsen et al., 2007). By detecting even smaller quantities of this protein, it is possible to identify patients who are at an early risk of developing some form of cardiovascular illness (Tiwari et al., 2012). The low limit of troponin-T detection achieved in this study provides a strong case for further investigation of the biosensing potential associated with zinc oxide.

An immunoassay was characterized for its performance metrics using electrical impedance spectroscopy (EIS) due to its capacity

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for distinguishing changes occurring at the electrical double layer (Randviir and Banks, 2013). EIS has previously been used for the detection of cardiac troponin-T biomarkers, although most do not achieve the detection ranges required for rapid diagnostics of cardiac events. Several researchers have utilized nanostructures with a wide range of physical geometries and electron confinement strategies for addressing this need (Karimian et al., 2013; Qureshi et al., 2012; Silva et al., 2010). These nanostructure-based sensor platforms require complex nanofabrication techniques which significantly add to the complexity of the device and the cost of the sensor. The biosensor presented here provides benefit over existing devices by achieving detection in the femtogram per milliliter (fg/mL) regime. It is fabricated using sputter deposition on a printed circuit board (PCB) substrate which is common to microelectronic device manufacturing. Functionalization of immunoassays on the nanotextured ZnO thin film allowed for specific detection of troponin-T at concentrations appropriate for assessing early risk of cardiovascular disease.

2. Materials and methods

Radio frequency (RF) magnetron sputtering at room temperature was used for the deposition of ZnO thin films on top of PCB substrates with patterned gold electrodes. This particular electrode design has been utilized previously with nanoporous alumina to detect various biomarkers at clinically relevant concentrations (Panneer Selvam and Prasad, 2012; Prasad et al., 2011).

2.1. Sensor platform

An organic PCB with two electroplated gold electrodes was used as the substrate for ZnO thin film deposition. All electrodes were 1 mm wide with 1 mm gaps between each edge. The working and reference electrodes were patterned on the PCB in an interdigitated concentric circular design as shown in Fig. 1(a). Short leads were soldered to each of the electrodes, allowing for connection of the platform to a potentiostat. A polydimethylsiloxane (PDMS) encapsulating manifold was adhered to the PCB with the ZnO thin film using a silicon adhesive sealant. The opening in the PDMS manifold allowed for fluid injection and containment on the metal sensing site. The assembled sensor can be visualized in Fig. 1(b).

Zinc oxide was selectively deposited onto the electrode regions of the PCB substrates using a 99.999% target from Kurt J. Lesker Co. in an ATC Orion RF magnetron sputter system by AJA International. Fig. 1(a) shows the transparent metal oxide film deposited on the small sensing portion of the PCB substrate. Sputtering was performed at room temperature with a 12 sccm of total gas flow rate and pressure of 5–15 mTorr. Sputter deposition parameters of power 140 and 200 W (W) and oxygen flow rates 0 and 2 sccm were investigated to identify the ideal electrical characteristics and surface morphology for optimizing the platform's biosensing capability. Argon flow rates were adjusted to either 12 or 10 sccm depending on the oxygen flow rate. The differing conditions for each sputter run were as follows: 140 W-without O_2; 140 W-with O_2; 200 W-without O_2; 200 W-with O_2.

2.2. Surface characterization

Following the deposition of ZnO on the PCB substrates, film thickness measurements were obtained using a Veeco Dektak 8 profilometer. The sheet resistance was then measured using an Alessi 4-point probe connected to a Keithly 2400 source meter, which allowed for resistivity calculations for each sputter condition. The surface morphology was visualized by performing tapping mode atomic force microscopy (Veeco Model 3100 Dimension V) on a 2 μm × 2 μm portion of the nanotextured ZnO film.

2.3. Immunoassay protocol for troponin-T detection

2.3.1. Sensitivity assay

The immunoassay was prepared by first immobilizing a cross-linking molecule to the sensing site. This was performed using a 10 mM solution of dithiobis succinimydyl propionate (DSP) dissolved in dimethyl sulfoxide (DMSO). After 30 min room temperature incubation, excess DSP was washed with DMSO. Next, a 100 ng/mL sample of anti-troponin-T capture antibody dissolved in phosphate buffer saline (PBS) was added onto the sensing site. Following another 15 min incubation and wash with PBS, Superblock was added to the sensor and left to incubate for 15 min to block any unoccupied N-hydroxysuccinimide sites on the DSP molecules.

The troponin-T detection steps began by first adding a sample of PBS without any solute onto the sensing site. At this point, an impedance measurement was taken using a Gamry Reference 600 potentiostat. After removal of the PBS, the lowest concentrated sample of troponin-T (10 fg/mL) was placed on the sensor. The antigen was allowed to incubate for 15 min before another set of impedance measurements were taken. Five additional dilutions of troponin-T, ranging from 100 fg/mL to 1 ng/mL, were subsequently added to the sensor with impedance measurements being taken for each sample. Wash steps were completed between each sample using PBS. A schematic of the entire immunoassay setup can be visualized in Fig. 1(d). The immunoassay preparation and troponin-T detection procedure were completed in triplicate for each of the four separate ZnO sputter conditions. Each of the experimental repeats was completed on independent sensors on different days within one week of sputter. Sensors were stored in a closed plastic container inside a standard laboratory cabinet at room temperature to show stability of the ZnO films.

2.3.2. Specificity assays

The robustness of the sensor was evaluated in two separate ways. First it was tested for the ability to specifically detect the presence of troponin-T. This was accomplished by preparing the immunoassay on the ZnO sensor surface using the exact same procedure previously stated. However, instead of testing for dilutions of troponin-T, bovine albumin solutions were applied to the sensor using the same concentration range. Next, the sensor was tested for potential use in biological media. Again, the immunoassay was prepared the same as before, but for this experiment, troponin-T was dissolved in human serum instead of PBS. Both of these experiments provide insight into the sensor's applicability as a point-of-care diagnostic tool.

2.4. EIS for biosensing

The performance of the ZnO sensor platform was tested by attempting to detect the biomarker troponin-T using electrical impedance spectroscopy. After adding each consecutive antigen sample to the sensing site, impedance measurements were taking by applying a small 10 mV AC potential to the PCB at frequencies ranging from 100 mHz to 1 MHz. The resultant impedance (Z) was calculated at each frequency with the real part (Z_{real}) of the impedance relating to the resistance in the system and the imaginary part (Z_{imag}) of the impedance relating to the capacitance in the system.

Biomolecule detection can be interpreted based on the change in impedances of the real and imaginary parts of the system as modifications occur at the electrical double layer (E_{dl}) formed at the fluid–metal oxide interface. When a fluid is dispersed on the surface of the sensor under an applied potential, there exists an